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Mechanisms of Alcoholic Liver Disease: Cytokines

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This article represents the proceedings of a workshop at the 2000 ISBRA Meeting in Yokohama, Japan. The chair was Manuela G. Neuman. The presentations were (1) New aspects of hepatic fibrosis, by D. A. Brenner; (2) Cellular immune response in hepatitis C models, by B. Rehermann; (3) The role of interleukin-10 in acute alcoholic hepatitis, by J. Taieb, S. Chollet-Martin, M. Cohard, J. J. Garaud, and T. Poynard; (4) Cytokine-mediated apoptosis in vitro, by M. G. Neuman; (5) Signaling for apoptosis and repair in vitro, by G. G. Katz, R. G. Cameron, N. H. Shear, and M. G. Neuman; (6) Interferons activate the P42/44 mitogen-activated protein kinase and Janus Kinase signal transducers and activation of transcription (JAK-STAT) signaling pathways in hepatocytes: Differential regulation by acute ethanol via a protein kinase C-dependent mechanism, by B. Gao; (7) Genetic polymorphisms of interleukin-1 in association with the development of Japanese alcoholic liver disease, by M. Takamatsu, M. Yamauchi, M. Ohata, S. Saito, S. Maeyama, T. Uchikoshi, and G. Toda; and (8) Increased levels of macrophage migration inhibitory factor in sera from patients with alcoholic liver diseases, by T. Kumagi, S. M. F. Akbar, M. Abe, K. Michitaka, N. Horiike, and M. Onji.

Key Words: Hepatic Fibrosis, Hepatitis C, Interleukin-10, Protein Kinase C, Alcoholic Liver Disease.

DR. NEUMAN DISCUSSED her laboratory's interest in the role of cytokine in mediating ethanol (EtOH)-induced apoptosis in vitro. A combination of low doses of EtOH (the equivalent of the level found in human blood after one or two glasses of wine) with acetaminophen (the equivalent of two Tylenol tablets) induces tumor necrosis factor (TNF)-α-dependent hepatocyte apoptosis that resembles immune-mediated fulminant hepatic failure in humans. Intracellular pathways that originate at the TNF-α receptor are linked to apoptosis (Neuman et al., 1999). Focusing on the TNF-α-dependent pathways by using an in vitro model of normal human hepatocytes, Dr. Neuman offered the view of a basic scientist who was investigating the mechanisms of EtOH-induced liver damage at the molecular and ultrastructural levels. Hepatocytes exposed to high but clinically relevant doses of EtOH revealed the same morphological features that can be observed in patients with alcoholic liver damage: steatohepatitis, megamitochondria, enlargement of endoplasmic reticulum, and intracellular Mallory-like bodies. Some cells underwent apoptosis, whereas other cells underwent necrosis or aponecrosis. Transmission electron microscopy and DNA fragmentation have been used to demonstrate the role of anti-TNF-α in blocking apoptosis processes. The activation of TNF-α-dependent pathways may be modulated in vitro and may have implications in the development of new therapeutic strategies to prevent hepatic injury (Neuman et al., 1993, 1995).

Studying the pathways of signaling for apoptosis and repair, in vitro, Dr. Katz evaluated whether polyenylphosphatidylcholine (PPC) affects apoptosis in a co-culture of normal human stellate cells with HepG2. PPC was postulated by Mi et al. (2000) to protect against cirrhosis in alcohol-fed baboons by preventing the associated hepatic phosphatidylcholine depletion. By using transmission electron microscopy and terminal transferase-mediated deoxyuridine 5-triphosphate nick end labeling assays, the author was able to show that PPC significantly down-regulates apoptotic processes previously unregulated by dose level and the frequency of exposure to EtOH via inhibition of caspase 3 and stabilization of permeability pore transition pathways (Katz et al., 2001).

Hepatic stellate cells not only are contributors to hepatic

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metabolism of alcohol but are the major source of extracellular matrix proteins in hepatic fibrosis, which include type I collagen. Dr. Brenner spoke about the new aspects of hepatic fibrosis. In response to liver injury, the hepatic stellate cells change from a quiescent to an activated phenotype. This activation process includes a phenotypic change to a myofibroblast-like cell, increased proliferation rate, loss of retinoid stores, and increased production of extracellular matrix proteins, chemokines, cytokines, and contractility. Ongoing studies are characterizing the genes that are differentially expressed in the quiescent and activated hepatic stellate cell. Dr. Brenner's group also investigated the regulation of type I collagen expression, the cleavage of collagen propeptides, and the formation of collagen cross-links. Dr. Brenner's group has demonstrated that a key regulatory step in hepatic fibrosis is the increased stability of the collagen α1(I) mRNA. Understanding these pathways may provide new insights into the molecular pathogenesis of hepatic fibrosis (Lang and Brener, 1999; Lindquist et al., 2000a,b; Stefanovic et al., 1999).

Due to the awareness of viral hepatitis impact on the progression of alcoholic liver disease (ALD), Dr. Rehermann spoke about cellular immune responses in hepatitis C. Hepatitis C virus (HCV) has a remarkable ability to establish clinically persistent infections in individuals who are otherwise immunocompetent. A strong T helper cell (Th) 1 dominant response targeted against immunodominant and highly conserved epitopes in structural and nonstructural protein of the virus has been demonstrated in patients with acute, self-limited hepatitis C. Lack of such Th response in the early phase of infection is associated with chronic evolution of infection. Similarly, the intrahepatic cytotoxic Th response is weaker in chronic HCV. The emergence of viral mutants or quasispecies with sequence variations in T-cell epitopes may play a role, as demonstrated in HCV-infected patients and in chimpanzees. There is also increasing evidence that several HCV proteins such as core E1/E2 and NS5A interfere with an efficient immune response. Progression to persistent infection as well as immunological mechanisms of liver injury is the consequence of complicated interactions between virus and host. Fortunately, the generation of full-length, stable, and infectious HCV molecular clones has allowed researchers to prospectively study virus-host interaction and role of drugs during the early phase of hepatitis C in an animal model of infection (Liang et al., 2000; Rehermann, 1999, 2000; Rehermann and Chisari, 2000).

The concept of anti-inflammatory cytokine therapy is not unique to alcohol-induced liver disease. Successful human clinical trials that involve other diseases such as rheumatoid arthritis and inflammatory bowel disease, in which TNF-α is increased, are already under way. However, with cytokine and anticytokine antibody therapy, there is a potential to impair liver regeneration by completely inhibiting TNF-α and interleukin (IL)-6 activity. Dr. Taieb spoke about the role of IL-10 in acute alcoholic hepatitis (AAH).

Features of the acute phase response and liver neutrophil infiltrate accompany AAH. Plasma levels of proinflammatory cytokines TNF-α and IL-6 and IL-8 are increased markedly in AAH and closely correlate with disease severity (Taieb et al., 1998). The authors measured circulating levels of IL-10, IL-8, and TNF-α in plasma and liver tissue samples in three patient populations, which included cirrhotic patients with severe AAH (Maddrey $>$32) treated by Prednisolone (40 mg/day, 28 days). The authors compared the obtained levels with the levels of the same end points in alcoholic cirrhotic patients without AAH and in healthy volunteers' blood samples. Cytokines were assayed in peripheral blood. At day 0, the circulating level of IL-8 was increased markedly in AAH as compared with cirrhotic patients with no AAH (5-fold) and with healthy volunteers (50-fold). IL-8 and TNF-α tissue levels were elevated although not correlated with plasma levels, because tissue IL-10 was undetectable. At day 14, IL-8 decreased significantly whereas TNF-α remained elevated and IL-10 increased at day 21; moreover, IL-10 production by blood monocytes from AAH patients seemed to increase after 28 days of corticosteroid therapy. Dr. Taieb concluded that the low concentration of IL-10 in the plasma and the liver of AAH patients may suggest that the initial anti-inflammatory response is defective. Recombinant human IL-10 (Tenovil) is a cytokine with anti-inflammatory and immunomodulatory properties that may have a beneficial effect in AAH by down-regulating proinflammatory cytokines.

Next, Dr. Gao continued to explore the use of cytokines as therapy and why this therapy is less efficient in alcoholic patients with chronic hepatitis. Interferons (IFN) are cytokines that are secreted endogenously. In patients with HCV that cured spontaneously, the level of IFN was found to be elevated, which is the rationale for use of this cytokine as therapy. Gao reports that IFN gamma rapidly activate the JAK-STAT1 signaling pathway and p42/44 mitogen-activated protein kinase in freshly isolated rat hepatocytes. EtOH markedly inhibits IFN gamma induced STAT1 activation and tyrosine phosphorylation (Chen et al., 1999; Nguyen and Gao, 1999). Blocking protein kinase C activation partially prevents ethanol inhibition of IFN-induced STAT1 activation, which suggests that protein kinase C may be involved. The author suggested that by inhibiting cellular mechanisms, EtOH may provoke unresponsiveness of IFN therapy in alcoholics.

Only 20% of the subjects who drink the same amount of alcohol for the same period of time will develop alcohol-induced liver injury. What make these people prone to liver damage? What differentiates them from the other 80%? Dr. Takamatsu described the association between the genetic polymorphism IL-1 in the development of ALD in the Japanese population. Only a minority of alcoholics ultimately develop alcoholic liver cirrhosis and alcoholic hepatitis due to chronic abuse that lasts over years or decades, so it is presumed that genetic factors may be involved. The

levels of IL-1 are elevated in patients with ALD, especially in those with cirrhosis and alcoholic hepatitis. Recently, the presence of genetic polymorphisms of this cytokine was confirmed (Takamatsu et al., 1998). The aim of this study was to determine whether IL-1 polymorphisms are associated with the development of ALD. Dr. Takamatsu and colleagues examined the frequency of two polymorphisms in the IL-1 gene located in promoter −511 and exon 5 +3953 locus by restriction fragment length polymorphisms in 142 male patients with ALD, 30 heavy drinkers without ALD, and 218 healthy controls. The carriers of −511 IL-1 allele 2 and heterozygotes of +3953 polymorphism were both significantly higher in heavy drinkers without ALD than in patients with ALD. The haplotype, IL-1 −511 allele 2/+3953 allele 1, was associated with the development of alcoholic cirrhosis. Dr. Takamatsu concluded that IL-1 polymorphisms may be related to the development of ALD in Japanese alcoholics.

Dr. Kumagi gave an interesting insight into the role of macrophage migration inhibitory factor (MIF). MIF plays an important role during host immune response to stress, infection, inflammation, and endotoxin via autocrine/paracrine and endocrine routes. Dr. Kumagi reported the increased level of MIF in sera from 30 patients with ALD and in 25 healthy normal subjects. The levels of MIF in sera in ALD patients was two times higher than in normal subjects but did not show any correlation with the levels of transaminases in sera. Dr. Kumagi concluded that MIF might play a complex role in the pathogenesis of ALD. Discussions stressed the importance of the immune system in ALD and the interest of researchers and physicians in understanding the clinical and basic science associations. Because of the hectic schedule and because another workshop was scheduled after our workshop, Dr. Neuman invited the audience to continue discussing specific technical problems with the four young presenters who gave short oral and poster presentations. The posters were displayed in the same room as the workshop so longer discussion and clarifications were available.

Dr. Neuman closed the workshop by noting the diversity of topics considered during the scientific sessions and by offering her view on the information that had emerged concerning the role of cytokines in both experimental and clinical inflammation. In view of the striking negative interaction between alcoholic liver injury and hepatitis C, an antiviral agent that is not contraindicated in alcoholic patients is needed. Anti-inflammatory cytokines as well as anti-inflammatory agents also may be useful.

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